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Short communication

Evaluation of techniques for the recovery of live intestinal *Trichinella spiralis* worms from experimentally infected foxes

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Abstract

Previously described methods for the recovery of intestinal *Trichinella* worms from rodents are not feasible when applied in larger experimental animals such as foxes. In this study, worm recovery by standard technique of simple incubation of the intestine in saline was compared to embedment of the intestine in an agar gel. The small intestines of *Trichinella spiralis* infected foxes (4–5 days post inoculation) were slit lengthwise and the two corresponding halves were processed with one of the two incubation methods. Worms were recovered from all samples, and the total worm recovery ranged from 0.2–4.4% of the infection dose. The samples from the standard incubation were very unclear and time consuming to count compared with samples from the agar gel embedment, in which the intestinal debris were kept inside the agar. As the agar gel technique generally yielded higher numbers of worms than the corresponding standard incubation sample, it is with some optimisation, recommended for recovery of intestinal *Trichinella* worms from foxes.

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Most comparative work on in vivo biological characteristics of *Trichinella* spp. and host reactions has been conducted in laboratory rodents (Pozio et al., 1992; Li et al., 1999; Li and Ko, 2001; Goyal et al., 2002). Rodent models are popular due to the low cost,

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availability, and convenient housing facilities, but results obtained in such systems may not entirely reflect characteristics of importance for the natural transmission of the parasite. Members of the genus *Trichinella* infect a broad range of animals (Kapel, 2000) but are essentially co-evolutionary adapted to the physiology of carnivorous hosts and several epidemiological studies conducted in Europe point to especially foxes as the main reservoir (Pozio, 1998). Experimental studies in both omnivorous and herbivorous animals have shown considerable variation in infectivity of the different *Trichinella* species, but for carnivores infectivity appears to be more uniform (Kapel, 2000). For epidemiological and taxonomical purposes it is therefore important that studies on the basic biology of *Trichinella* aim to use epidemiologically relevant species, e.g. carnivores such as foxes.

For the recovery of intestinal worms, previously described methods are time consuming and not convenient when applied to larger animals, especially in comparative studies where several animals need processing within short time. Thus, for studies on intestinal establishment, population dynamics, and cross breeding of different *Trichinella* species in carnivores, development of alternative methods is essential.

The described techniques for recovering intestinal worms are either 4–5 h long incubation of rinsed pieces of intestine in 37 °C NaCl saline (Blair, 1983) or incubation of intestines in a thermal migration chamber with a heat gradient as described by Despommier (1973), a method based on the thermophilic behaviour of the worms. According to Blair (1983), the worms migrate to the intestinal lumen soon after the death of the host and it is therefore essential to rinse and incubate the intestines within minutes. This rapid procedure is not practically possible in extensive experiments with larger animals, and the rinsing procedure is not recommendable if intestines cannot be processed immediately after the host is killed. Consequently, samples originating from intestines that are not rinsed are unclear and difficult to count.

In the present study, the standard technique of simple incubation in saline was compared with a method where the intestinal mucosa or the whole intestine was embedded in an agar gel and the worms were allowed to migrate through the agar into 0.9% saline. This method leaves debris from the intestinal contents and tissue inside the agar. The use of agar to hold back unwanted debris was developed to recover nematode larvae from herbage samples (Jørgensen, 1975; Mwegoha and Jørgensen, 1977). Later van Wyk et al. (1980) modified the method to recover nematodes from gastrointestinal contents of sheep and it has furthermore been modified to recover nematode larvae from various tissue homogenates: *Oesophagostomum dentatum* in pigs, *Ascaris suum* in pigs and mice (Slotved et al., 1996a,b, 1997a,b; Murrell et al., 1997; Saeed et al., 2001).

Four adult female farm foxes were each inoculated orally with 14,000 *Trichinella spiralis* (Code: ISS 004 at the *Trichinella* Reference Centre, Rome, Italy) muscle larvae recovered from mice by digestion (Kapel and Gamble, 2000). Two foxes were killed 4 days post infection (dpi) and the remaining two foxes five dpi. Euthanasia was performed by intravenous injection of 0.5 ml/kg Pentobarbital (20%). The foxes were fed ad libitum immediately after infection and then deprived food until necropsy to reduce the amount of intestinal content. Water was allowed ad libitum throughout the experiment. The gastrointestinal tracts were removed at necropsy, kept warm (30–37 °C) in therm boxes and transported to the laboratory within 2–4 h. Because of this long transportation time the intestines were not rinsed as this would discharge any worms that had already moved into

the lumen. The small intestine was divided in an upper and a lower section. Each of these sections was cut down the middle lengthwise to obtain two equally large halves. From the upper intestinal section, one half was hung over a hook in a conical glass with 300 ml 0.9% saline (37 °C), the other half was placed mucosa upwards on a humid cloth in a low horizontal tray. One end of the cloth was rolled around a stick and fastened with clips. Subsequently, 100 ml of 2% agar solution (45 °C) was mixed with 100 ml 0.9% saline (37 °C), poured carefully over the intestine and allowed to solidify. The cloth with the agar gel was then removed from the tray and hung in a plexiglass chamber (23 cm × 34 cm × 6 cm) containing 4.5 l 37 °C saline. From one half of the lower section the mucosa was scraped off into 100 ml 0.9% saline (37 °C) saline and mixed 100 ml 2% agar (45 °C), poured onto a humid cloth and treated as above. The other half was incubated directly in saline as above. All samples were left for three hours at 37 °C and then transferred a new chamber with fresh saline and left overnight. After incubation, the samples were allowed to settle for 20 min before the supernatants were removed by suction and the sediments were poured into conical glasses, allowed to settle, washed twice and finally poured into 50 ml tubes. The samples were allowed to sediment 20 min between washes. To examine if all worms sedimented within 20 min, the supernatants from two foxes (fox 3 and 4, [Table 1](#)) samples were passed through a sieve with a 20 µm pore size and the residues were washed into 50 ml tubes. To evaluate the number of worms from the other gastrointestinal sections, the stomach and large intestine of two foxes were cut open and hung separately in 0.9% saline (37 °C) overnight before being treated as above. All samples were counted using a stereo microscope (40× magnification).

Worms were recovered from all four foxes both from the upper and lower sections of the small intestine. The total worm recovery ranged from 0.2 to 4.4% with the highest recovery (622 worms) from fox no. 3 five dpi ([Table 1](#)). The agar method with whole intestinal pieces embedded in the agar gel (treatment 2) yielded the most worms from the upper section in three foxes (no. 1, 2 and 3), whereas only five worms in total were found from the upper section of a single fox (no. 4) and four of the worms were in the standard incubation sample. In the lower intestinal sections, the agar method (treatment 4) gave highest worm numbers in three foxes (no. 2, 3, and 4); only for one fox (no.1) higher numbers obtained with the standard incubation method.

In general, the direct incubation of intestinal pieces in saline resulted in very unclear samples, especially from overnight incubation. Agar-embedding of both whole intestinal sections and intestinal scrapings resulted in more clear samples, which were 5–10 times faster to count than samples from the standard incubation (10–30 min compared with 2–6 h). Additionally, the agar samples yielded either more worms or comparable number of worms to the standard incubation in saline. A fast procedure is essential in many types of experiments, and it is therefore important information that the majority of the worms (59–91%) could be harvested after 3 h. Depending on the logistics involved in an experiment a longer harvesting time can be chosen with an accordingly higher recovery of worms. The overall worm recovery was surprisingly low. Nevertheless recovery percentages have been seen to vary widely both within and between experiments and range from 2.8% in guinea pigs ([Roth, 1938](#)), 5–19% in rats ([Nuñez et al., 2002](#)), 3–62% in mice (a.o. [Sukhdeo and Meerovitch, 1980](#); [Bell et al., 1985](#); [Rossi et al., 1994](#); [Goyal et al., 2002](#)) and 5–22% in pigs ([Murrell, 1985](#); [Marti and Murrell, 1986](#)). Also the time from

Table 1
Recovery of adult *Trichinella spiralis*, from experimentally infected foxes, by different techniques

Necropsy	Fox no.	Section of small intestine	Treatment method		No. of worms	Total no. worms	Recovery from each section (%)	Total recovery of inoculated dose/fox (%)
Day 4	1	Upper	1. Standard incubation	3 h + overnight	31 + 7	38	22	2.7
		Upper	2. Agar whole intestine	3 h + overnight	109 + 27	136	78	
		Lower	3. Standard incubation	3 h + overnight	61 + 67	128	63	
		Lower	4. Agar mucosa	3 h + overnight	27 + 48	75	37	
	2	Upper	1. Standard incubation	3 h + overnight	0 + 0	0	0	0.2
		Upper	2. Agar whole intestine	3 h + overnight	8 + 3	11	100	
		Lower	3. Standard incubation	3 h + overnight	3 + 0	3	25	
		Lower	4. Agar mucosa	3 h + overnight	9 + 0	9	75	
Day 5	3	Upper	1. Standard incubation	3 h + overnight	103 + 54	157	37	4.4
		Upper	2. Agar whole intestine	3 h + overnight	260 + 8	268	63	
		Lower	3. Standard incubation	3 h + overnight	60 + 18	78	40	
		Lower	4. Agar mucosa	3 h + overnight	119 + 0	119	60	
	4	Upper	1. Standard incubation	3 h + overnight	3 + 1	4	80	0.2
		Upper	2. Agar whole intestine	3 h + overnight	1 + 0	1	20	
		Lower	3. Standard incubation	3 h + overnight	1 + 1	2	7	
		Lower	4. Agar mucosa	3 h + overnight	27 + 0	27	93	

necropsy till harvest procedure is known to lower the recovery of other intestinal nematodes using the agar embedment technique (Nosal et al., 1998) and further development of a satisfying method for recovery intestinal *Trichinella* should therefore also focus on reducing the transport time prior to actual processing of the intestines.

Worms recovered from the colon of foxes no. 3 and 4 corresponded to 9% (57 worms) and 23% (8 worms) of the total recovery for these animals. However, it is not known whether this reflects mucosal establishment or post mortem dislocation. The gut should therefore be divided in the desired sections immediately after the host is killed.

Since pooled supernatants from foxes no. 3 and 4 contained all together 149 worms, it is concluded that not all worms sediment within 20 min. The number of worms in the supernatants was low in the treatments where many worms were recovered but high, compared with the total number of worms, in the treatments where few worms were found. This implies that information on worm numbers may be lost with the supernatants in low grade infections. This observation leads us to suggest that samples should be sieved, a procedure which would also reduce the time needed for the whole processing.

The simple methodology, with clean recovery, and easy enumerable samples make the agar gel method superior for recovery of live *T. spiralis* from fox intestines. Unless a different method to scrape the mucosa is applied, the embedding of the whole intestine must be considered the best method, especially combined with sieving instead of sedimentation of samples.

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